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ORIGINAL COMMUNICATION

Dietary and serum α-tocopherol, β-carotene and retinol, and risk for colorectal cancer in male smokers

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Objective: To study the association between dietary and serum antioxidant vitamins and carotenoids and risk for colorectal cancer in male smokers.
Design: A prospective cohort study within a randomised, double-blind, placebo-controlled trial testing supplementation with α-tocopherol (50 mg/day), β-carotene (20 mg/day) or both in preventing cancer.
Subjects and methods: Participants of the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study with complete dietary data and serum samples available from baseline. These included 26 951 middle-aged male smokers among whom 184 colorectal cancer cases were diagnosed during 8 y of follow-up. Relative risks were calculated with Cox proportional hazards models adjusting for trial supplementation, age, body mass index, serum cholesterol, cigarettes smoked per day and physical activity.
Results: There was no significant association between dietary vitamin C or E, α-or γ-tocopherol, retinol, α- or β-carotene, lycopene or lutein + zeaxanthin and risk for colorectal cancer. Serum α-tocopherol, β-carotene or retinol was also not associated with the risk, neither did the season when baseline blood was drawn modify the relationship between serum β-carotene and colorectal cancer risk.
Conclusions: Our data support the results from previous studies in which no association between dietary antioxidant vitamins and carotenoids and risk for colorectal cancer has been observed. Likewise, no association between baseline serum antioxidant concentrations and colorectal cancer risk was evident.
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Introduction

There are over 850 000 new cases of colorectal cancer annually, worldwide (Cannon, 1997). The incidence of colon cancer is similar in both men and women, but rectum cancer is more common in men. Established risk factors include age, genetic predisposition and long-term smoking. Nutritional factors affecting the process are largely open, but epidemiological studies have indicated consumption of vegetables and grains to be inversely associated with risk for colorectal cancer (Cannon, 1997). This may be due to specific compounds found in grains and vegetables, such as fibre, vitamins, minerals and possibly other anticarcinogenic factors. On the other hand, many studies have found foods rich in antioxidants rather than selected food components to be inversely associated with the risk. The evidence concerning carotenoids has been extensively reviewed, and no consistent association between colorectal cancer and carotenoids has been found (World Health Organization, 1998a). Likewise, there is no consistent evidence of any association between antioxidant vitamins and colorectal

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cancer (Heilbrun *et al*, 1989; Willett *et al*, 1990; Bostick *et al*, 1993), and studies on serum or plasma vitamin concentrations and colorectal cancer have not shown significant associations (Comstock *et al*, 1992; Longnecker *et al*, 1992).

The aim of this study was to investigate the association between dietary antioxidant vitamins and carotenoids and serum α-tocopherol, β-carotene, and retinol concentration and risk for colorectal cancer in a prospective cohort of middle-aged male smokers.

Methods

This study was done as part of the Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) Study (The ATBC Cancer Prevention Study Group, 1994). The ATBC Study was a randomised, double-blind, placebo-controlled primary prevention trial conducted to determine whether supplementation with α-tocopherol (50 mg/day), β-carotene (20 mg/day) or both could prevent cancer. The participants were all male smokers (at least five cigarettes/day) at study entry, aged 50–69 y, and residents of southwestern Finland. Men who at baseline reported a history of cancer or had severe diseases limiting long-term participation ($n = 2560$), or who took supplements of vitamins E (>20 mg/day) or A ($>20\,000$ IU/day) or β-carotene (>6 mg/day; $n = 184$), were excluded. In all, 29 133 men entered the trial, which ended in April 1993. Thereafter, follow-up for cancers, other diseases and deaths has continued through national registries.

Data collection

At study entry, all participants completed a questionnaire about sociodemographic factors and general medical history, and each gave a fasting serum sample, which was stored at -70°C. Serum cholesterol concentration was determined enzymatically by the CHOD-PAP method (Kattermann, 1984) within 2–4 y after baseline.

Serum α-tocopherol, β-carotene and retinol concentrations were determined by high-performance liquid chromatography (HPLC) assay (Milne & Botnen, 1986) within 2–4 y after serum collection. A reversed-phase column was used for the simultaneous determination of the vitamins by using isocratic elution with methanol as the single eluant. A diode-array detector was used to monitor the elution at 292 nm for α-tocopherol, at 325 nm for retinol, and at 450 nm for β-carotene. The peak heights were used in the calculations. All manipulations were carried out in yellow light in order to avoid photo-isomerisation of the compounds. The between-run coefficients of variation were 2.2% for α-tocopherol, 3.6% for β-carotene and 2.4% for retinol.

Diet was assessed using a self-administered modified diet history method (Pietinen *et al*, 1988). The questionnaire included over 200 food items and over 70 mixed dishes and it was filled in with help from a portion size picture booklet. The subject was asked to report the usual frequency of consumption and the usual portion size of foods during the previous 12 months. The questionnaire was satisfactorily completed by 27 111 participants (93%) at baseline. Dietary intake of nutrients was calculated by use of the software and food-composition data available at the National Public Health Institute of Finland. The dietary questionnaire was compared with food records in a separate validity study of 190 middle-aged men, and the reproducibility of vitamin assessment was studied in 121 men who completed the food-use questionnaire three times at three-month intervals (Pietinen *et al*, 1988). Correlations between nutrient intake values from the food records and the food-use questionnaire ranged from 0.41 for vitamin A to 0.64 for vitamin E. The intraclass correlations from the three food-use questionnaires varied from 0.56 for vitamin A to 0.70 for vitamin E.

Colorectal cancer

Incident colorectal cancer cases (ICD9-codes 153- and 154-) were identified through the Finnish Cancer Registry and the Registry of Causes of Death. Colorectal cancer diagnosis was confirmed centrally by review of the hospital records and of histopathological specimens. Among the 26 951 trial participants with all baseline data available (among these dietary data and serum values), 184 colorectal cancer cases, excluding carcinoid tumour and anal cancers, were diagnosed by 30 April 1995. The median follow-up time for the cohort was 8.0 y (interquartile range 1.6 y).

Statistical analyses

Cox regression models were used to estimate the association between dietary antioxidant vitamins and carotenoids, and serum concentrations of α-tocopherol, β-carotene and retinol and the risk for colorectal cancer. Our analysis used follow-up time starting from randomisation and ending at diagnosis of colorectal cancer, at death, or at the end of follow-up (30 April 1995). Dietary variables were log-transformed and energy-adjusted according to the Willett residual method (Willett & Stampfer, 1986). Dietary and serum variables were entered into the models as indicator variables defined by the second through fourth quartiles among the entire cohort, with the lowest quartile as the reference group. An ordinal score variable was also created to test for dose-response relationships across levels of dietary and serum variables.

In multivariate models we adjusted for baseline age, body mass index (BMI), number of cigarettes smoked per day, occupational and leisure-time physical activity, serum cholesterol concentration, alcohol intake and study supplementation. Most of these factors (age, serum cholesterol, BMI, number of cigarettes smoked per day) were significantly related to colorectal cancer risk in our study population, and others (alcohol, physical activity) were of borderline significance. Total years of smoking was strongly correlated with baseline age and was not associated with colorectal cancer risk in the multivariate model. Thus, we did not include total years of smoking in the final model. The trial supplementation group was included in the models because of a suggestion for a protective effect for α-tocopherol supplementation (Albanes *et al*, 2000). Exclusion of cases found during the two first years had no essential effect on the results.

Because serum β-carotene has been shown to vary by season in this cohort (Rautalahti *et al*, 1993), we also tested the interaction between serum β-carotene and season (categorised by baseline blood sampling into two seasons: high concentrations between August and December and low concentrations between January and July) on colorectal cancer risk. We also tested the interaction between serum β-carotene (categorised into low and high) and β-carotene supplementation and serum α-tocopherol (categorised into low and high) and α-tocopherol supplementation by having the main effects and their cross-product terms in the models simultaneously. The interaction between smoking and the dietary and serum variables studied was also tested using pack-years of smoking as the dosage of tobacco consumption (as indicator variables defined by quartiles among the entire cohort).

Results

Participants developing colorectal cancer during follow-up were older at baseline and had, consequently, smoked for a longer time than had non-cases (Table 1). Likewise, the cases smoked more cigarettes per day at baseline than did non-cases, even if the median number of cigarettes per day was identical. Cancer cases had a lower serum cholesterol level than non-cases, but regarding baseline BMI, education, total energy and alcohol intake, and serum α-tocopherol, β-carotene and retinol concentrations, cancer cases and non-cases were similar. The cases lived in big cities more often than non-cases. Leisure time physical activity was similar among cases and non-cases, but cases were more often not working at baseline. Of those still working at baseline, cases had more often sedentary jobs than had non-cases, as previously reported (Colbert *et al*, 2001).

Table 1 Baseline characteristics (medians or proportions) of colorectal cancer cases and non-cases

	Cases	*Non-cases*
Number of subjects	184	26 767
Age (y)	60.3	57.1
Total years of smoking	40	36
Number of cigarettes/day	20	20
Body mass index (kg/m^2)	26.3	26.0
Total energy intake (kcal/day)	2736	2720
Alcohol intake (g/day)	10.7	11.0
Serum cholesterol (mmol/l)	5.87	6.17
Serum alpha-tocopherol (mg/l)	11.7	11.5
Serum beta-carotene (μg/l)	173	172
Serum retinol (μg/l)	577	577
Dietary alpha-tocopherol (mg/day)	9.3	9.2
Dietary beta-carotene (μg/day)	1818	1713
Dietary retinol (μg/day)	1263	1247
Married (%)	77	81
Living in a big city (%)	52	42
Education (%)		
> 11 y	11	10
7–11 y	25	25
< 7 y	65	65
Physically active during leisure time (%)	57	59
Occupational physical activity (%)		
Not working	54	42
Sedentary	33	33
Active	13	26

In the entire cohort of 26 951 men, baseline serum α-tocopherol was strongly correlated with serum cholesterol ($r=0.60$), but less so with vitamin E intake ($r=0.26$) and BMI ($r=0.15$). Serum β-carotene was positively correlated with serum cholesterol ($r=0.17$) and with β-carotene and α-carotene intake ($r=0.23$ and $r=0.21$, respectively), but negatively with alcohol intake ($r=-0.20$). Serum retinol was also positively correlated with serum cholesterol ($r=0.22$) and alcohol intake ($r=0.20$), but only weakly inversely with retinol intake ($r=-0.06$). These correlations were statistically significant.

We observed no associations between dietary intakes of antioxidant vitamins including carotenoids and colorectal cancer risk (Table 2). Multivariate models gave essentially similar risk estimates, as did the models with age-adjustment only. There were no significant associations between baseline serum α-tocopherol, β-carotene or retinol and colorectal cancer risk (Table 3). Season had no modification effect on the association between serum β-carotene and colorectal cancer risk. We did not observe any meaningful modification effect for trial α-tocopherol or β-carotene supplementation on the association between serum α-tocopherol or β-carotene concentration and risk for colorectal cancer, respectively. Neither was there any interaction between smoking and the dietary and serum variables studied.

Discussion

We found no significant associations between prospectively ascertained dietary antioxidant vitamins or carotenoids and colorectal cancer. Moreover, serum α-tocopherol, β-carotene or retinol concentrations were not associated with colorectal cancer incidence.

The association between antioxidants and colorectal cancer was studied in all supplementation groups combined, since the placebo group had insufficient cases for comparison. However, study supplementation was included in the multivariate models, and no interaction was observed between study supplementation and dietary or serum α-tocopherol or β-carotene. Even if known confounders were adjusted for in the analyses, there may still have been other unknown confounding factors, as well as residual confounding for the factors included in the models. There was also a potentially increased need for dietary antioxidants in the

Table 2 Energy-adjusted nutrient intake quartiles and relative risk (RR) for colorectal cancer ($n = 184$) with 95% confidence intervals (95% CI) in a cohort of middle-aged male smokers ($n = 26\,951$)

	Quartile group, median intake				
Nutrient	*1*	*2*	*3*	*4*	P *for trend*
Vitamin C (mg/day)	59.1	84.7	110	153	
Number of cases	44	47	41	52	
Multivariate[a] RR	1.00	1.08	0.93	1.16	0.64
95% CI		0.72–1.64	0.60–1.43	0.77–1.76	
Vitamin E (mg/day)	7.50	9.30	11.7	17.7	
Number of cases	43	47	39	55	
Multivariate RR	1.00	1.14	0.94	1.26	0.42
95% CI		0.75–1.73	0.60–1.46	0.83–1.89	
α-Tocopherol (mg/day)	6.50	8.00	10.0	15.1	
Number of cases	42	48	37	57	
Multivariate RR	1.00	1.19	0.91	1.33	0.32
95% CI		0.78–1.80	0.58–1.43	0.88–2.00	
γ-Tocopherol (mg/day)	1.90	4.30	7.80	16.7	
Number of cases	42	43	46	53	
Multivariate RR	1.00	1.05	1.12	1.26	0.26
95% CI		0.68–1.61	0.73–1.72	0.83–1.90	
Retinol (μg/day)	657	979	1476	2433	
Number of cases	53	34	52	45	
Multivariate RR	1.00	0.65	1.02	0.86	0.92
95% CI		0.42–1.01	0.70–1.50	0.58–1.29	
α-Carotene (μg/day)	21.2	53.8	99.0	198	
Number of cases	42	44	48	50	
Multivariate RR	1.00	1.05	1.12	1.11	0.57
95% CI		0.68–1.60	0.74–1.71	0.73–1.69	
β-Carotene (μg/day)	839	1370	2085	3620	
Number of cases	39	42	59	42	
Multivariate RR	1.00	1.08	1.48	1.06	0.49
95% CI		0.70–1.68	0.98–2.23	0.68–1.64	
Lycopene (μg/day)	146	432	772	1456	
Number of cases	39	51	52	42	
Multivariate RR	1.00	1.32	1.32	1.06	0.82
95% CI		0.87–2.00	0.87–2.02	0.68–1.66	
Lutein + zeaxanthin (μg/day)	998	1255	1481	1841	
Number of cases	48	35	53	48	
Multivariate RR	1.00	0.75	1.11	1.00	0.59
95% CI		0.48–1.16	0.75–1.65	0.66–1.50	

[a]Adjusted for age, body mass index, alcohol intake, serum cholesterol, physical activity, cigarettes/day, and trial supplementation.

present cohort due to smoking. Additional methodological limitations include those common to nutritional epidemiology, like measurement error, high correlations between nutrient intakes, and the fact that dietary intake may not reflect tissue or serum concentrations accurately.

The absence of any association between α-tocopherol intake and risk for colorectal cancer is in line with most previous studies. In two large female cohorts including both smokers and non-smokers, the Iowa Women's Health Study and the Nurses' Health Study, no significant association was observed between dietary intake of vitamin E and colorectal cancer risk (Willett *et al*, 1990; Bostick *et al*, 1993; Sellers *et al*, 1998). Case–control studies have yielded conflicting results: either inverse associations (Ferraroni *et al*, 1994; Ghadirian *et al*, 1997; La Vecchia *et al*, 1997) or no association has been observed (Freudenheim *et al*, 1990; Benito *et al*, 1991; Peters *et al*, 1992; Meyer & White, 1993; Olsen *et al*, 1994; Slattery *et al*, 1998). The intake level of dietary vitamin E in the present cohort was similar to that reported from other study populations (Peters *et al*, 1992; Slattery *et al*, 1998), and the intake level varied sufficiently: there was a 2.5-fold difference in median intake of vitamin E between the first and fourth quartile. A recent review concluded that γ-tocopherol may be superior to α-tocopherol in increasing the antioxidant status of ingested food (Stone & Papas, 1997), but we also found no association between dietary γ-tocopherol and colorectal

Table 3 Baseline serum vitamin quartiles and relative risk (RR) for colorectal cancer ($n=184$) with 95% confidence intervals (95% CI) in a cohort of middle-aged male smokers ($n=26\,951$)

Serum factor	*Quartile group, median concentrations* 1	2	3	4	P *for trend*
α-Tocopherol (mg/l)	8.61	10.7	12.5	15.4	
Number of cases	49	42	56	37	
Age-adjusted RR	1.00	0.85	1.15	0.77	0.53
95% CI		0.57–1.29	0.78–1.69	0.50–1.18	
Multivariate RR[a]	1.00	0.90	1.30	0.94	0.72
95% CI		0.59–1.39	0.85–2.00	0.57–1.57	
β-Carotene (μg/l)	79	141	210	354	
Number of cases	51	41	52	40	
Age-adjusted RR	1.00	0.77	0.95	0.71	0.23
95% CI		0.51–1.16	0.65–1.40	0.47–1.08	
Multivariate RR[a]	1.00	0.83	1.08	0.86	0.82
95% CI		0.54–1.26	0.72–1.63	0.54–1.36	
Retinol (μg/l)	452	540	616	734	
Number of cases	46	45	51	42	
Age-adjusted RR	1.00	1.01	1.18	1.03	0.69
95% CI		0.67–1.53	0.79–1.76	0.68–1.57	
Multivariate RR[a]	1.00	1.02	1.19	1.02	0.75
95% CI		0.67–1.54	0.79–1.79	0.65–1.58	

[a]Adjusted for age, body mass index, alcohol intake, serum cholesterol, physical activity, cigarettes/day, and trial supplementation.

cancer. Moreover, α-tocopherol supplementation (50 mg daily) had no statistically significant effect on colorectal cancer in the ATBC Study (Albanes *et al*, 2000).

Baseline serum α-tocopherol was not associated with colorectal cancer in the present study. A pooled analysis of five cohorts with 289 cases and 1267 matched controls suggested an inverse association between serum α-tocopherol concentration and colorectal cancer, but this result was attenuated by adjustment for serum cholesterol (Longnecker *et al*, 1992). The number of cases in the individual studies varied from 23 to 108, and the sera were stored between 0 and 14 y before analysis. In the present study the serum samples were stored for less than 2 y before analysing and the storage temperature was -70°C, sufficiently low for the antioxidant content to be stable (Comstock *et al*, 1993). In the present study serum α-tocopherol levels were slightly lower than in cohorts from other countries (Willett *et al*, 1984), but the difference was minimal. There was also enough variation in the ATBC cohort over the serum α-tocopherol quartiles since the highest quartile median was twice that of the lowest one.

The absence of any association between β-carotene intake and the risk for colorectal cancer in the present study is in line with findings from other prospective studies showing no significant associations between dietary carotenoids and colorectal cancer risk (Willett *et al*, 1990; Sellers *et al*, 1998; World Health Organization, 1998a). In about half of the case–control studies, dietary carotenes have been inversely associated with colorectal cancer risk (van Poppel, 1993; Ferraroni *et al*, 1994; La Vecchia *et al*, 1997). In such studies, however, carotene intake may have been altered, either due to changes in dietary habits or changes in metabolism caused by the disease. The intake of carotenoids was lower in our study population compared to other cohorts: the median intake of β-carotene was less than 2 mg/day in the present cohort, although with substantial variation, as compared to between 3 and 6 mg/day in other populations (Enger *et al*, 1996; Sellers *et al*, 1998; Tucker *et al*, 1999; Slattery *et al*, 2000). This lower intake level might be partly due to the fact that the participants were all middle-aged male smokers having a relatively low consumption of fruit and vegetables.

The intakes of other carotenoids, including α-carotene, lycopene, lutein and zeaxanthin and retinol were not associated with colorectal cancer. In contrast to our finding, lutein has been inversely associated with colon cancer in a large case–control study from California (Slattery *et al*, 2000). Data on specific carotenoids remain sparse, however.

Serum β-carotene was not associated with colorectal cancer in the present study. This is in line with results from numerous other studies where no significant associations have been observed between pre-diagnostic blood levels of carotenoids and colorectal cancer (Schober *et al*, 1987; Connett *et al*, 1989; World Health Organization, 1998a). Nor have large, controlled trials demonstrated protective effects for β-carotene supplementation in colorectal cancer (Hennekens *et al*, 1996; Omenn *et al*, 1996; Albanes *et al*, 2000).

We observed no association between dietary or serum retinol and colorectal cancer. Little evidence from other epidemiological studies suggests that retinol (preformed vitamin A) may be protective against colorectal cancer. This is not surprising, since both dietary and serum retinol are poor markers to reveal any role for retinol in tumorigenesis among normally nourished people (World Health Organization, 1998b). Dietary retinol is primarily stored in the liver, and due to an elaborate homeostatic control mechanism, serum retinol concentrations remain fairly constant over a wide range of dietary intake and total body reserves of vitamin A.

In conclusion, we found no evidence that dietary or serum antioxidant vitamins or carotenoids were associated with risk for colorectal cancer in a large cohort of male smokers.

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